

Analogy between the oral microbial flora found at the level of carious lesions, endodontic spaces and prosthetic components

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Abstract

Nowadays, eating habits and lifestyles are moving towards a diet high in sugars and carbohydrates, which is extremely harmful to both oral integrity and systemic health. The aim of the study was to identify the variations between the bacterial plaque formed in carious lesions, infected root canals and prosthetic components, in comparison with commensal oral microflora. The study was carried out on a group of 21 patients aged between 7 and 84 years, 13 women and 8 men, with various oral diseases, which were diagnosed during the specialized examination. The preparation of the medical record, the signing of the informed consent, the anamnesis and the consultation were carried out in the private dental office where the patients presented for a personalized treatment plan, and the examination of the pathological products and the interpretation of the results obtained in the Microbiology Discipline, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania. The study carried out on the preparations taken from patients with carious lesions reveals the presence of numerous G+ cocci arranged in short chains (23%), frequent G+ cocci in diplo, thin G+ bacilli with rounded ends and G+ cocci in diplo, arranged in small piles (15%), and values lower than 8% are represented by G+ in diplo cocci, lanceolate, rare G+ in diplo cocci, very rare fusiform bacilli and dentinal fragments. The study carried out on the preparations taken from the endodontic space showed a remarkable percentage of epithelial cells and leukocytes (28%), rare fusiform bacilli, very small fragments of epithelial tissue, thin G+ bacilli with rounded ends, G+ cocci in diplo and in small piles and buns arranged in short chains (5-6%). The study carried out on the preparations taken from the level of prosthetic components, recorded a relatively high number of epithelial cells (19%), G+ cocci arranged in diplo, lanceolate and in small piles (13%), thin G+ bacilli, with rounded ends (10%), cocci in short chains (10%) and rare polymorphonuclear leukocytes (10%), while at the opposite pole, squamous epithelial cells, fusobacteria and pleomorphic forms (3%) are found.

Keywords: Microbiological examination; Oral microbial flora; Carious lesion; Endodontic space; Prosthetic components

1. Introduction

Nowadays, eating habits and lifestyles are moving towards a diet high in sugars and carbohydrates, which is extremely harmful to both oral integrity and systemic health [1-5].

Through the intake of carbohydrates in large quantities, carious disease appears, one of the most frequent pathologies of the oral cavity in recent decades [6-9]. Untreated carious processes cause, most of the time, irreversible changes on the dental tissues, and thus patients can present from a specific endodontic pathology, to complicated carious processes, accompanied by partial or total edentations [10-17].

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The aim of this work was to identify the variations between the bacterial plaque formed in carious lesions, infected root canals and prosthetic components, in comparison with commensal oral microflora.

2. Materials and methods

2.1. Materials

The following materials were used:

- Sterile degreased glass slides,
- Physiological serum,
- Alcohol 90%,
- Pathological products collected from patients,
- Sterile disposable consultation kit,
- Sterile cotton rolls,
- Oral aspirator,
- Sterile exploratory probes,
- Bacteriological loops,
- Special Gram kit for staining slides,
- Optical microscope with immersion objective (x100),
- Immersion Oil (Cedar Wood Oil),
- Bunsen bulb,
- Camera of the Samsung Galaxy A40 phone.

2.2. Methods

2.2.1. Selection of the patient group

The study was carried out on a group of 21 patients aged between 7 and 84 years, 13 women and 8 men, with various oral diseases, which were diagnosed during the specialized examination.

The preparation of the medical record, the signing of the informed consent, the anamnesis and the consultation were carried out in the private dental office where the patients presented for a personalized treatment plan. The examination of the pathological products and the interpretation of the results were carried out in the Microbiology Discipline, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania.

2.2.2. Collection of pathological products

For the collection of biological samples, sterile single-use consultation kits were used. The actual sampling of the pathological product from the level of the carious processes to obtain the altered dentine and from the surface of the prosthetic components, for the bacterial plaque, was done with the help of exploratory probes, and in the case of samples taken from the infected root canals, paper cones were used.

2.2.3. Preparation of a slide smear

The step to obtain high-quality smears was as follows:

- degreasing glass slides with 70% alcohol
- distribution of a drop of physiological serum in the center of the blade
- displaying the pathological product:
 - carefully unloading the biological sample from the exploratory probe onto the slide
 - the bacteriological loop was sterilized in the flame of the Bunsen bulb by moving back and forth
 - with the help of the sterile loop, concentric movements are made from the center of the blade outwards until the product is formed in as thin a layer as possible on the blade
- placing the blade to dry until a whitish film is observed
- fixing the product by passing the blade through the flame of the Bunsen bulb 3 times
- staining of smears was done by the Gram technique, which is a double stain in which Gram-positive bacteria are stained with gentian violet and then decolorized with alcohol-acetone mixture.

Reagents needed for Gram staining:

- Crystal violet
 - Lugol's solution
 - Acetone/ethanol mixture
 - 0.1% basic fuchsin solution
 - Water
- Gram staining technique:
 - applied 3-4 drops of gentian violet on dry and fixed smear, then waited 3 minutes
 - the dye was removed and 2-3 drops of Lugol's were applied for 1 minute
 - Lugol's solution was removed, we decolorized the slide with an alcohol-acetone mixture
 - the blade was washed under the tap
 - the slide was restained with fuchsin diluted 1/10 and waited 1-2 minutes
 - the blade was washed for the last time under the jet
 - the blade was placed in a special support for drying
 - Microscopic examination of slide
 - The x100 immersion objective was used, after previously applying a drop of cedar oil to the slide. The slide was fixed on the microscope stage and the x100 objective was lowered until it contacted the slide. The fields to be photographed were clarified with the help of the microvisa, after which their detailed analysis and interpretation followed.

3. Results and Discussion

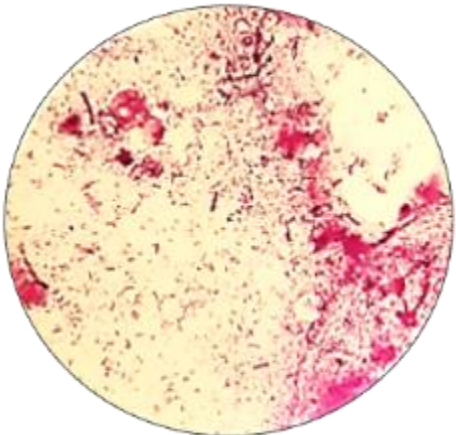
The study was conducted on a group of 21 patients, 13 female and 8 male, aged between 7 and 84 years.

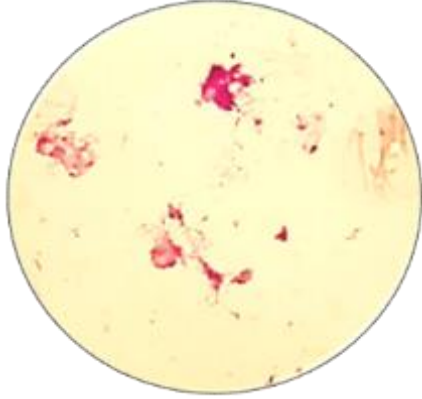
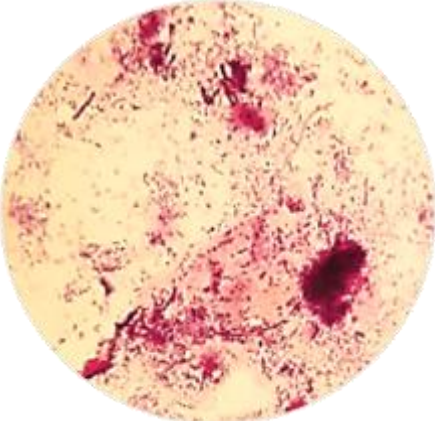
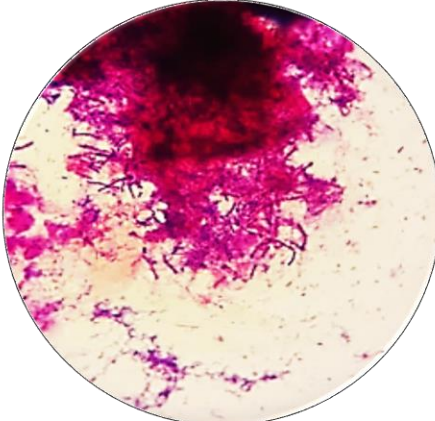
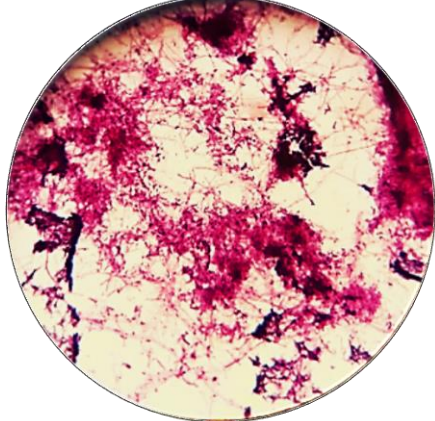
They come from both urban and rural areas, with a preponderance of those living in urban areas, the degree of oral hygiene being fluctuating, from good oral hygiene, to unsatisfactory.

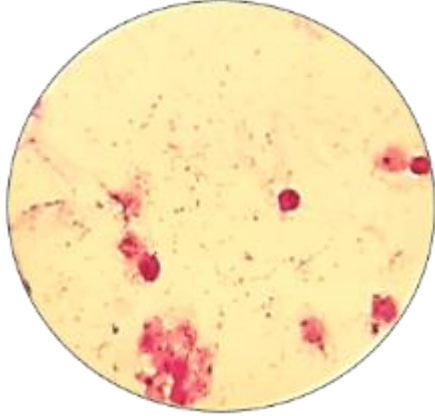
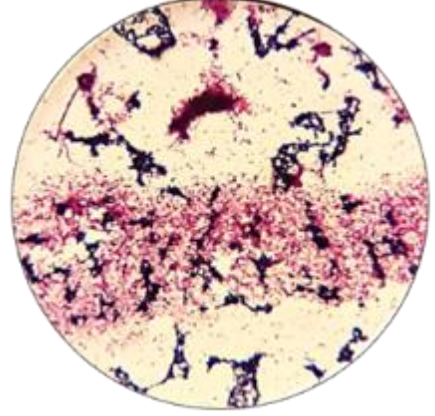
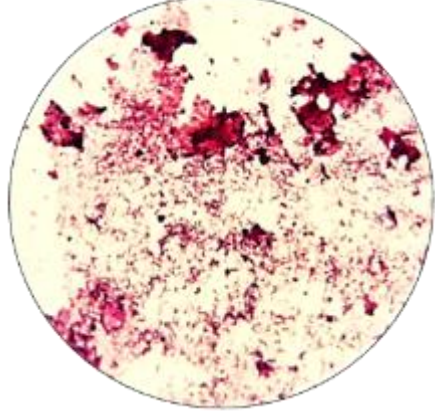
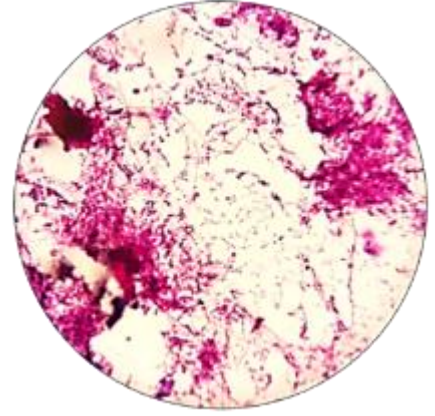
The patients presented various associated pathologies, and following the anamnesis, clinical and radiological examination, various conditions in the oral cavity were detected, such as carious processes, acute inflammation of the dental pulp or pulp necrosis.

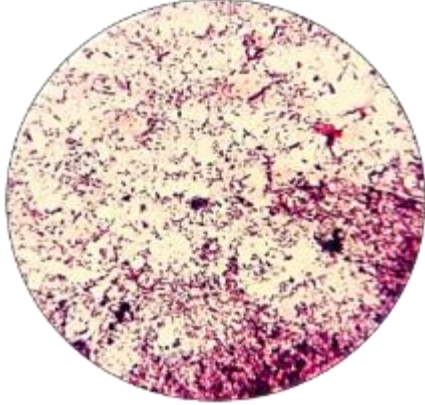
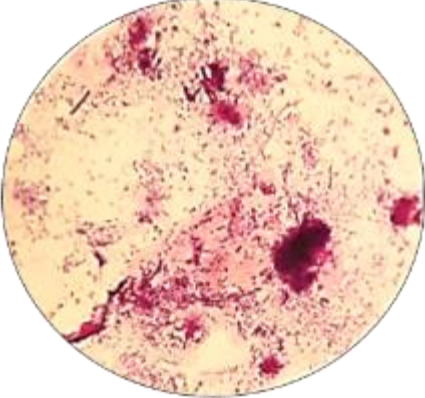
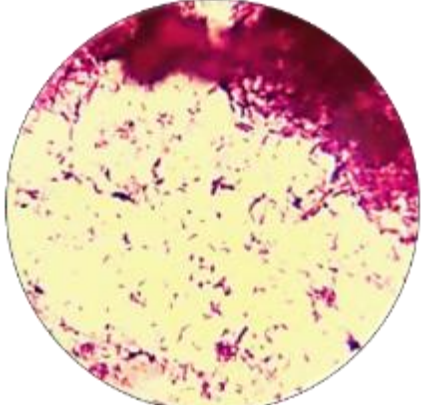
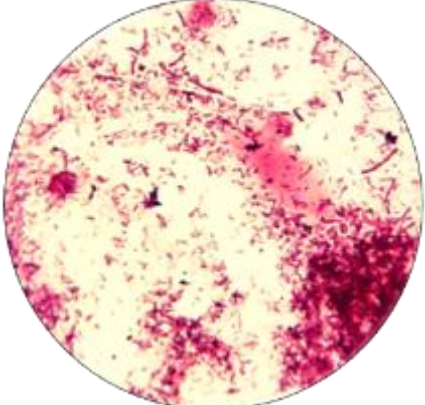
After examining ten successive smear fields, the most representative image was chosen, and interpretation was made after estimating the microbial ratio on all 10 fields (Table 1).

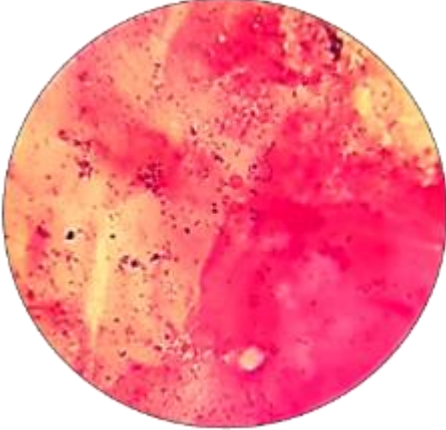
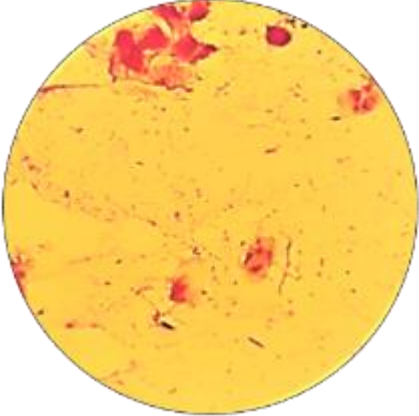
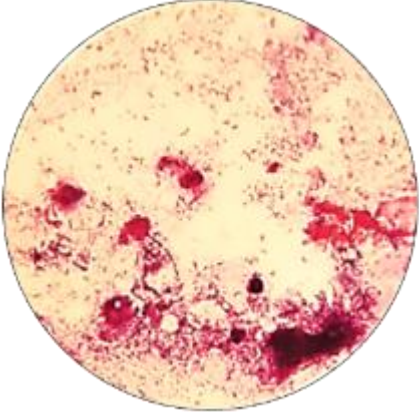
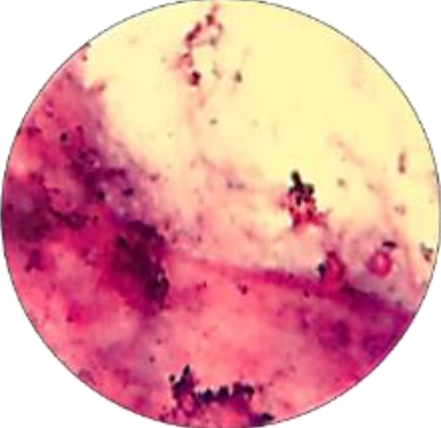
Table 1 Data obtained from examination of smears for each patient

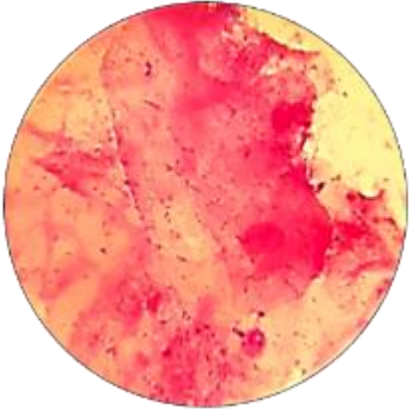
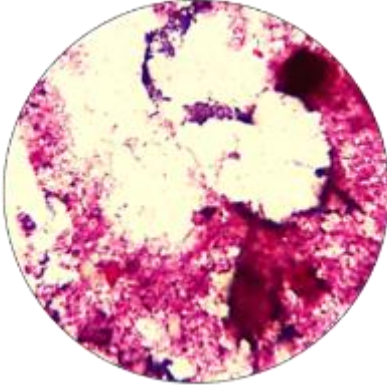
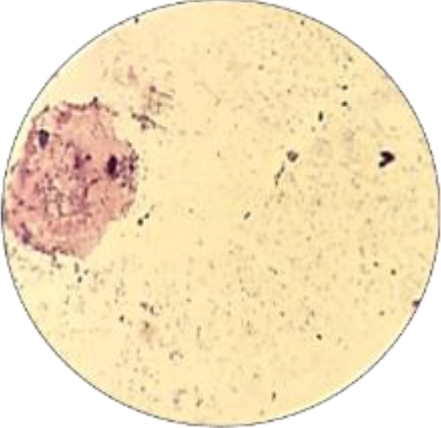
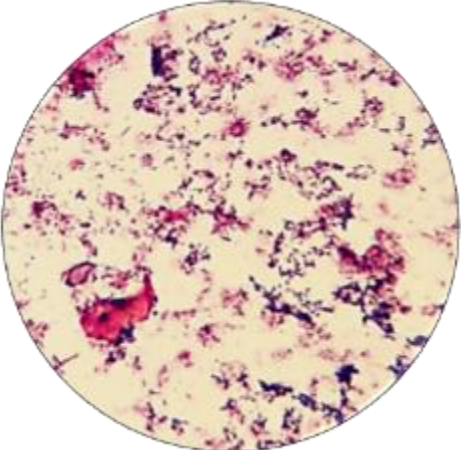
No. crt.	Smear image	Smear interpretation
1.		<ul style="list-style-type: none"> - epithelial cells - thin G+ bacilli with rounded ends - G+ buns in diplo, in small piles - cocci G+ in diplo, lanceolate

<p>2.</p>		<ul style="list-style-type: none"> - very small fragments of epithelial tissue - 1-2 G+ cocci
<p>3.</p>		<ul style="list-style-type: none"> - very rare polymorphonuclear leukocytes (PMNs) - rare epithelial cells - round G+ cocci, lanceolate, in diplo, in small and somewhat larger piles - few cocci in short chains - rare longer and thicker G+ bacilli
<p>4.</p>		<ul style="list-style-type: none"> - balanced flora G+ and G- - G+ cocci in chains, dominant over G+ in diplo - rare G- thin bacilli - desquamated epithelial cells
<p>5.</p>		<ul style="list-style-type: none"> - frequent fusobacteria - G+ cocci in diplo, isolated - rare shells G- - pleomorphic forms

6.		<ul style="list-style-type: none">- 4 leukocytes per field- 2-3 epithelial cells per field- rare G+ cocci in diplo
7.		<ul style="list-style-type: none">- very frequent G+ cocci in diplo- G+ bacilli, with heads rounded in equal proportion to the cocci
8.		<ul style="list-style-type: none">- dentine fragments- numerous G+ bacilli with rounded heads- rare G+ cocci arranged in diplo, lanceolate and round
9.		<ul style="list-style-type: none">- G+ cocci isolated or in diplo- very rare cocci in chains- very small piles of G+ cocci in diplo- rare epithelial cells

10.		<ul style="list-style-type: none">- an equal ratio, with the slight dominance of G+ bacilli over G+ cocci, arranged in isolation or in diplo
11.		<ul style="list-style-type: none">- very rare PMNs- rare epithelial cells- G+ cocci round, lanceolate, in diplo, in small and larger piles- few cocci in short chains- rare longer and thicker G+ bacilli
12.		<ul style="list-style-type: none">- G+ bacilli predominate- G+ cocci in diplo
13.		<ul style="list-style-type: none">- G+ cocci, abundant in diplo, rare in chains, rare small piles- very rare G+ bacilli with rounded ends

<p>14.</p>		<ul style="list-style-type: none"> - rare cocci in diplo - G+ cocci in heaps, relatively frequently - epithelial cells
<p>15.</p>		<ul style="list-style-type: none"> - G+ cocci in diplo relatively present, very rare lanceolate in diplo - very rare G+ bacilli with rounded ends
<p>16.</p>		<ul style="list-style-type: none"> - PMNs - rare epithelial cells - diplo cocci, with short chains, grouped in small piles - rare G+ bacilli, thin, with rounded ends - cocci G+ in diplo, lanceolate, grouped in short chains
<p>17.</p>		<ul style="list-style-type: none"> - epithelial cells - G+ cocci in diplo and small piles - very rare fusiform bacilli

18.		<ul style="list-style-type: none">- epithelial cells- G+ cocci in diplo, in small piles- rare cocci G+ in diplo, lanceolate
19.		<ul style="list-style-type: none">- rare epithelial cells- G+ cocci in diplo, in piles and short chains- a G- fusiform bacillus
20.		<ul style="list-style-type: none">- 1-2 epithelial cells per field- rare G+ cocci in diplo and lanceolate
21.		<ul style="list-style-type: none">- frequent G+ cocci in small piles, and rare larger piles- cocci in short chains

The results of the detailed analysis of the elements observed on the smears examined in our study are presented in the following table (Table 2).

Table 2 Analysis of the incidence of the elements observed on the microbiological preparations in the studied group

Samples from carious lesions	Periodicity of the appearance of biological elements in the analyzed samples	Samples from the endodontic spaces	Periodicity of the appearance of biological elements in the analyzed samples	Samples from the surface of prosthetic components	Periodicity of the appearance of biological elements in the analyzed samples
Epithelial cells	-	Epithelial cells	5	Epithelial cells	6
Rare PMNs	-	Rare PMN	-	Rare PMN	3
Leukocyte	-	Leukocyte	4	Leukocyte	-
G+ cocci in diplo, in small piles	2	G+ cocci in diplo, in small piles	1	G+ cocci in diplo, in small piles	4
G+ cocci in diplo, lanceolate	1	G+ cocci in diplo, lanceolate	3	G+ cocci in diplo, lanceolate	4
Cocci in short chains	3	Cocci in short chains	1	Cocci in short chains	3
G+ cocci in diplo, isolated	-	G+ cocci in diplo, isolated	-	G+ cocci in diplo, isolated	2
Frequent G+ cocci in diplo	2	Frequent G+ cocci in diplo	-	Frequent G+ cocci in diplo	-
Slender G+ bacilli with rounded ends	2	Slender G+ bacilli with rounded ends	1	Slender G+ bacilli with rounded ends	3
Rare G- thin bacilli	-	Rare G- thin bacilli	-	Rare G- thin bacilli	1
G+ bacilli	2	G+ bacilli	1	G+ bacilli	4
Very rare fusiform bacilli	1	Very rare fusiform bacilli	1	Very rare fusiform bacilli	-
Dentin fragments	1	Dentin fragments	-	Dentin fragments	-
Pleomorphic forms	-	Pleomorphic forms	-	Pleomorphic forms	1
Fusobacteria	-	Fusobacteria	-	Fusobacteria	1
Desquamated epithelial cells	-	Desquamated epithelial cells	-	Desquamated epithelial cells	1

4. Conclusion

The study carried out on the preparations taken from patients with carious lesions reveals the presence of numerous G+ cocci arranged in short chains (23%), frequent G+ cocci in diplo, thin G+ bacilli with rounded ends and G+ cocci in diplo, arranged in small piles (15 %), and values lower than 8% are represented by G+ cocci in diplo, lanceolate, rare G+ cocci in diplo, very rare fusiform bacilli and dentinal fragments.

The study carried out on the preparations taken from the endodontic spaces showed a remarkable percentage of epithelial cells and leukocytes (28%), rare fusiform bacilli, very small fragments of epithelial tissue, thin G+ bacilli with rounded ends, G+ cocci in diplo and in small piles, and cocci arranged in short chains (5-6%).

The study carried out on the preparations taken from the level of prosthetic components, recorded a relatively high number of epithelial cells (19%), G+ cocci arranged in diplo, lanceolate and in small piles (13%), thin G+ bacilli, with rounded ends (10 %), cocci in short chains (10%) and rare PMNs (10%), while at the opposite pole, squamous epithelial cells, fusobacteria and pleomorphic forms (3%) are found.

The microbiological examination is of real practical use in the dental office, because the real-time highlighting of the oral microbial flora helps in establishing the subsequent therapeutic strategy.

Compliance with ethical standards

Acknowledgments

We gratefully acknowledge the patients for giving us permission to conduct this study.

Disclosure of conflict of interest

The authors declare no conflict of interest.

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No funding was received for this study.

Authors' contributions

Authors CLD, ICM and OB contributed to this work in conceptualization, methodology, software, and formal analysis. CCA and SDA contributed in software, formal analysis, and data curation. CLD, ICM, CCA, SDA and OB contributed in validation, supervision, project administration. All authors read and approved the final version of the manuscript.

Statement of informed consent

Informed consent was obtained from the patients included in the study.

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